Review

Reviewing the low efficiency of protein utilization in heavy preruminant calves – a reductionist approach

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Abstract – The efficiency of protein utilization for growth in preruminant calves is decreasing with increasing body weight. In contrast to calves weighing less than 100 kg of body weight, heavy preruminant calves do not respond in protein retention to an increased intake of indispensable amino acids in dose-response studies. The marginal efficiency of protein utilization is low compared with pigs and milk-fed lambs at a similar stage of maturity. A reductionist approach was taken to perceive the potential mechanisms for the low protein utilization in preruminant calves. Neither an imbalance in the dietary protein to energy ratio nor a single limiting indispensable amino acid was responsible for the low efficiency. Also, amino acids were not specifically used to detoxify ammonia. Alternative hypotheses to explain the low efficiency are discussed and result in (i) a reduced post-absorptive supply of amino acids: e.g. by fermentation of milk in the (premature) rumen or preferential amino acid utilization by specific tissues; or (ii) a reduced post-absorptive amino acid utilization: e.g. by decreased insulin sensitivity, utilization of amino acids for gluconeogenesis or an asynchronous nutrient supply. In conclusion, several mechanisms for the low efficiency of protein utilization in heavy preruminant calves were excluded. Other physiological processes which are potentially involved remain to be studied, because the large potential for improving protein utilization in heavy preruminant calves asks for further exploration of their amino acid metabolism.

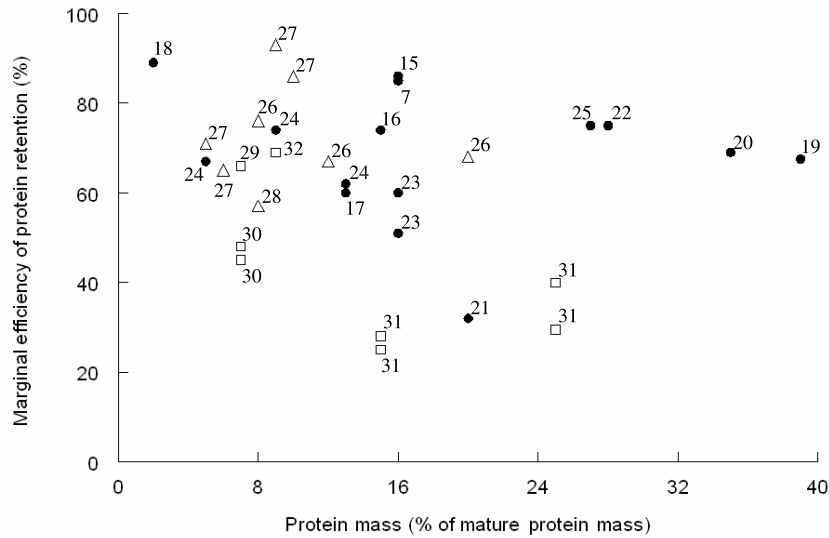calf / veal / protein metabolism / efficiency / amino acids / preruminant

1. INTRODUCTION

In contrast to the comprehensive work on protein digestion in milk-fed calves [1–5], the post-absorptive utilization of amino acids has only been scarcely investigated. This lack of attention is rather surprising, since only about 10% of the ingested nitrogen is lost with feces, and an additional 40–50% with urine. Furthermore, the efficiency

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Figure 1. Marginal efficiency of protein retention with increasing maturity in pigs (●) [15–25], preruminant lambs (△) [26–28], and preruminant calves (□) [29–32].

of protein utilization decreases with increasing body weight (BW) in preruminant calves. Although this reduction is in accordance with the decreasing amino acid utilization for protein accretion with increasing stage of maturity [6], the efficiency in heavy preruminant calves is extremely low compared with other species. In pigs, for example, gross efficiencies of protein utilization of 60% up to as high as 81% were reported [7–10]. For a sound comparison of efficiencies, however, different species should be compared at a similar stage of maturity, and marginal efficiencies, rather than gross efficiencies, should be compared. The marginal efficiency expresses the response of protein deposition rate to increased digestible protein intake, and consequently excludes digestion inefficiency and amino acid requirements for maintenance. It has the additional advantage of showing less between-experiment variation than the gross efficiency and is thus more suitable for comparison across literature sources.

Figure 1 shows the marginal efficiency with which digestible protein, or the first limiting amino acid, was used for protein gain in pigs, preruminant lambs and preruminant calves during (early) development. The protein mass, as the percentage of protein mass at maturity, is used to indicate the stage of maturity of each species. Assumptions for the mature protein mass were based on literature values for pigs [11], sheep [12, 13], and Holstein-Friesian bulls [14]. Marginal efficiencies vary substantially between studies and even within species at a similar stage of maturity. It is, however, illustrated that the marginal efficiency of protein retention is remarkably low in milk-fed calves as compared to pigs and milk-fed lambs. The differences are more pronounced with increasing protein mass. An exception in the figure are the control pigs of Krick et al. [15] which had a marginal efficiency of only 32%, but an energy constraint to protein deposition could not be excluded in that study.

This paper reviews the work, performed at the TNO Nutrition and Food Research Institute, ILOB, Wageningen, The Netherlands, over the last twenty years (in part unpublished), in which the low efficiency

of protein utilization in heavy preruminant calves was established. Following a reductionist approach and based on experimental evidence, various factors are excluded as reasons for the low efficiency. Alternative mechanisms are presented and discussed.

2. THE AGE DEPENDENCY OF PROTEIN UTILIZATION IN PRERUMINANT CALVES

Although Donnelly and Hutton [30] found a marginal efficiency of protein utilization of only 45% in young calves (40–70 kg BW), other studies reported substantially higher marginal efficiencies of 66–69% [29, 32]. Furthermore, from various sources [33–35], gross efficiencies of digestible protein utilization of 60–68% could be calculated for preruminant calves of similar BW.
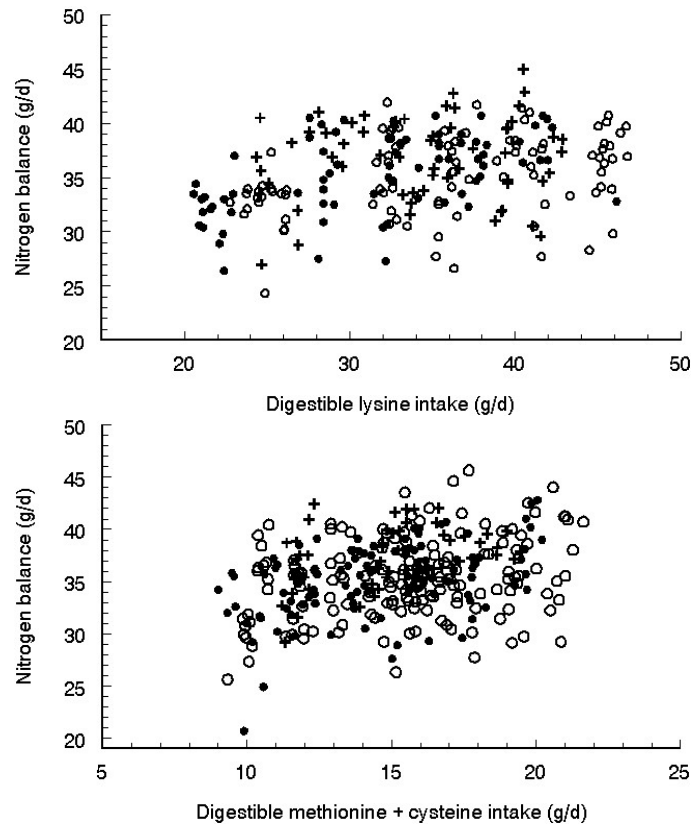
The clear response of protein gain to increased protein intake in young calves was confirmed in amino acid requirement studies [36, 37]. The amino acids studied were lysine, threonine and methionine+cysteine, and all experiments were performed with preruminant, male Holstein-Friesian × Dutch-Friesian crossbreeds, measuring nitrogen balance as the response variable to increased intakes of the amino acid of interest. Skimmed milk protein and crystalline amino acids were used as the only protein sources. The dietary crude protein content depended on the BW range of the calves and decreased from 220 $g \cdot kg^{-1}$ for calves between 50 and 70 kg BW to 180 $g \cdot kg^{-1}$ for the calves between 220 and 250 kg BW. Skimmed milk protein provided up to 160 $g \cdot kg^{-1}$ of the diet for calves between 50 and 70 kg, and up to 120 $g \cdot kg^{-1}$ for the calves between 220 and 250 kg. The remaining protein (60 $g \cdot kg^{-1}$) was provided as crystalline amino acids, except for the amino acid of the study which was added in graded levels. In total, crystalline amino acids provided maximally 70 $g \cdot kg^{-1}$ of the diet (a mixture of indispensable and dispensable amino acids). A quadratic response of nitrogen balance to increased intakes of lysine and methionine+cysteine was obtained for preruminant calves between 50 and 70 kg BW ($P < 0.01$) [38].

Similar studies were performed in heavy preruminant calves between 120 and 260 kg BW [36, 37]. Interestingly, responses of nitrogen balance to increased intakes of lysine and methionine+cysteine in heavy preruminant calves (120–260 kg BW) were absent (Fig. 2). Nitrogen balance only responded (slightly) to lysine intake in the range of 120–140 kg BW ($P < 0.05$). Possible explanations for the lack of response in heavy preruminant calves to an increasing amino acid intake include the following: (1) the use of large quantities of dispensable crystalline amino acids in these studies, and (2) a protein-energy imbalance in these amino acid requirement studies. Crystalline amino acids were included because skimmed milk powder was used as the main protein source, complicating a design of diets limited in specific indispensable amino acids. The possibility of an imbalanced protein to energy ratio was subsequently studied in serial slaughter experiments [31].

3. PROTEIN TO ENERGY IMBALANCE

In pigs, much more than in preruminant calves, research efforts have concentrated on the interaction between dietary protein and dietary energy [39–41]. At low protein intakes, growing pigs will preferentially use absorbed amino acids with a high efficiency for deposition in the body. With increasing intakes, protein retention will increase almost linearly up to the point at which other factors than protein intake limit protein retention. When maximum protein deposition has not been reached, energy intake is the limiting factor at this breakpoint in the curve. Any further increase in protein intake would not result in additional protein retention. Following the same approach for preruminant calves, two slaughter experiments were conducted to quantify

Figure 2. The effect of increased intake of lysine (top graph) and methionine + cysteine (bottom graph) on the N-balance of preruminant calves between 120 and 140 kg BW (●), 170 and 190 kg BW (○) and 240 and 260 kg BW (+). The top graph represents pooled data of nine trials ($n = 226$), the bottom graph pooled data of 13 trials ($n = 330$). Every symbol represents one observation, i.e. a 5-days' nitrogen balance of one calf. Data from [36, 37]. Nitrogen balance only responded to lysine intake in the range of 120–140 kg BW ($P < 0.05$).

the responses of protein and fat deposition rates to changes in protein and protein-free energy intake in preruminant calves from 80 to 240 kg BW [31]. Preruminant calves (90 male Holstein-Friesian × Dutch-Friesian crossbreeds) were fed a wide range of protein intakes (milk proteins only) at each of two protein-free energy intake levels.

Extra protein-free energy intake mainly results in extra fat deposition, but also increases protein deposition rate, even at low protein intakes [31]. This is in agreement with a study in ruminating steers receiving an abomasal infusion of amino acids and energy-yielding nutrients [42]. An increased protein intake results in extra fat and protein deposition [31], which corresponds with results in young preruminant calves (40–70 kg BW) [30]. Briefly, the results showed the absence of clearly distinguishable protein and energy dependent phases for protein deposition in calves, unlike the linear-plateau concept which is used for pigs. In addition, the efficiency of utilization of extra dietary protein was found to be very low: for every 100 g

increase in protein intake, protein deposition increased only with about 30 g [31]. It was concluded that the response of preruminant calves to increased nutrient intakes clearly differs from that of pigs, and that the lack of response of nitrogen balance in the amino acid requirement studies [36, 37] was not primarily caused by a limiting dietary energy supply.

4. AMINO ACID IMBALANCE

The possibility of a limiting indispensable amino acid causing both the low efficiency of nitrogen utilization in the slaughter trials [31] and the lack of response in the amino acid requirement studies [36, 37] still exists.

Gerrits et al. [43] presented the amino acid profiles in different body fractions and in the whole body of preruminant calves in the range of 80 to 240 kg BW as affected by protein and energy intake. For the detection of a possible dietary amino acid imbalance, they hypothesized that the marginal efficiency of utilization of at least one indispensable amino acid would be substantially higher than the reported marginal efficiency of 30% for total protein [43]. The marginal efficiencies found, however, were very low and within the rather narrow range of 11 to 29% for all indispensable amino acids [43]. Amongst the conditionally dispensable amino acids, arginine showed an efficiency of utilization of about 90%. The quantity of arginine ingested exceeded the quantity of arginine retained [43]. When compared with weaned pigs, which require at least 40% of the arginine requirement to be supplied by the diet, it seemed therefore unlikely that arginine was limiting protein deposition [43]. However, more recent studies suggest that arginine metabolism in neonatal, milk-fed animals may differ from that in weaned animals [44–46] (see Sect. 6.2), but it is not known if arginine supplementation affects protein deposition in heavy preruminant calves. The efficiency of utilization of cysteine was also high, on average 74%, while the efficiency of methionine was only about 27%. Utilization of methionine for the production of cysteine can not be excluded, but the relative increase in cysteine intake with increasing protein intake was low compared with other amino acids, which may have complicated the estimation of a marginal efficiency of cysteine [43]. In conclusion, not one of the indispensable amino acids limited the rate of protein deposition. This means that other reasons are responsible for the low efficiency of protein utilization in preruminant calves.

5. AMINO ACIDS FOR AMMONIA DETOXIFICATION

A temporary high portal flux of ammonia potentially stimulates amino acid utilization for ureagenesis [47]. In ureagenesis, mitochondrial ammonia and cytosolic aspartate are precursors for the ornithine cycle. Amino acids can be used as predominant N-donors for aspartate, which incurs a penalty on amino acids available for protein synthesis [48]. Degradation of amino acids to provide precursors for the ornithine cycle could (partly) explain an inefficient utilization of amino acids for protein gain. This was shown in short-term (2–3 h) studies in sheep [49] and dairy cows [50], although other studies indicate that ammonia-N may provide more than the theoretically expected 50% of the N-atoms in urea [51–53].

In preruminant calves, feeding diets based on either skimmed milk protein or a mixture of soy and wheat gluten (50/50) results in high portal ammonia fluxes, representing 9 to 19% of the dietary nitrogen intake (or 6 and 13 g ammonia-N$\cdot$d^{-1}) respectively [54]. Nutrient absorption and consequently portal nutrient and ammonia fluxes in preruminant calves are peak-wise [55], which indicates that high fluxes of ammonia are to be processed by the liver within a relatively short time-span. The ammonia may originate from intestinal amino acid deamination, but also from protein fermentation in the gastrointestinal tract. To quantify the contribution of amino

Table I. Least squares means of fecal and urinary nitrogen output and recovery of ^{15}N in preruminant calves at two levels of protein intake (58 vs. 83 g N·d^{-1}) and at two levels of ammonia infusion in the colon (0 vs. 10 g N·d^{-1}) with $^{15}NH_4Cl$ added as a tracer to the ammonia infusion. Data from [56] ($n = 12$ for each treatment).

| | Main effects[1] | | | |
	Low	High	SEM	P-value
Fecal N-output, g N·d^{-1}				
Protein intake	6.8	7.6	0.38	0.15
Ammonia infusion	6.9	7.4	0.23	0.18
Recovery ^{15}N in feces, %				
Protein intake	6.2	8.4	0.7	0.05
Urinary N-output, g N·d^{-1}				
Protein intake	27.1	42.5	0.95	< 0.001
Ammonia infusion	31.1	38.6	0.78	< 0.001
Recovery ^{15}N in urine, %				
Protein intake	48.5	53.9	1.97	0.07

[1] Two-way interactions were not significant ($P > 0.05$).

acids to the ammonia detoxification process, Gerrits et al. [56, 57] infused ammonia, as NH_4HCO_3, in the colon of preruminant calves with an average BW of 165 kg, at each of two protein intake levels (58 and 85 g N·d^{-1}; $n = 12$ for each treatment), and measured the increase of nitrogen losses compared with the infusion of $NaHCO_3$. Infusion rates were 10 g ammonia-N per day. Also, a tracer dose of $^{15}NH_4Cl$ was infused to estimate the kinetics and recovery of the infusate by analysis of ^{15}N enrichment in urine and faeces. Faeces and urine were separately and quantitatively collected during a 6-d balance period after an adaptation period to the dietary protein intake level (17 d) and to infusion into the colon (7 d). Calculated from the total nitrogen excretion, on average only 75% (variation between animals: 0–180%) of the infused ammonia-N was recovered in urine and no interaction with protein intake was found (Tab. I). Calculated from the tracer infusion, on average 51% (variation between animals: 36–63%) of the infused $^{15}NH_4Cl$ was recovered in urine. This implies that infused ammonia was only partially recovered in urine (from tracer calculations), and that the low net recovery of ammonia-N (collected up to 48 h after the end of infusion) could indicate a metabolic role of ammonia. The net recovery of infused ammonia-N was expected to exceed 100%. This would indicate extra amino acid catabolism. The low net recovery suggests, however, that no additional amino acids were catabolized to provide aspartate for the ornithine cycle. This could be due to the flexibility of the enzyme systems involved, i.e. glutamate dehydrogenase and carbamoyl-phosphate synthase 1/ornithine transcarbamylase, to supply aspartate and citrulline for urea synthesis respectively. In conclusion, it is unlikely that ureagenesis itself contributes to the inefficiency of nitrogen utilization in preruminant calves.

6. ALTERNATIVE MECHANISMS

From the work described above, it was concluded that marginal efficiencies of 30–35% are normal for preruminant calves

> 100 kg BW, using milk proteins as the sole protein source in the diet. Following a reductionist approach, it was shown that neither a protein-energy imbalance, an imbalanced amino acid profile nor the ureagenesis are responsible for the low marginal efficiency in heavy preruminant calves. Alternative hypotheses are presented and discussed below. Although individual mechanisms are described, the reason for a low efficiency of protein utilization in heavy preruminant calves may be multi-factorial in nature. Interactions between factors may be important to explain the decreased protein utilization. In order to identify the main effects and for the sake of simplicity, the mechanisms are discussed separately.

6.1. Fermentation of milk in the rumen

The estimation of the marginal efficiency of protein utilization may be complicated by ruminal drinking, certainly depending on the feeding method, but potentially also on feeding level and nutrient composition. Several studies describe the etiology and pathology of ruminal drinking [58–60], but studies reporting quantitative measurements of milk leakage into the rumen are scarce. Guilhermet et al. [61] and Wise et al. [62] used preruminant calves equipped with a rumen canula to estimate leakage of milk. Generally, both studies showed a large variation in the amount of milk recovered in the rumen between individual calves, which averaged ~ 7% [61] and 20% [62] of the milk ingested. Leakage considerably increases with age and is higher when animals are drinking from a bucket (~ 40%) than when sucking a nipple (< 1%) [61]. Tadeu dos Santos et al. [63], not mentioning the feeding method, reported 3% of spillage into the rumen in calves with a good appetite and 57% in calves with a reduced appetite. At our research station, Van Leeuwen [64] collected duodenal chyme after an oral dose of synthetic amino acids in milk and found a recovery of 84% in the duodenum. When amino acids are dissolved in water, only 10% is recovered in the duodenum [65],

illustrating a poor closure of the oesophageal groove after drinking of water. Since the quantity of milk appearing in the rumen, and nitrogen losses during (protein) fermentation are unknown, it is difficult to estimate the quantitative impact of ruminal drinking on the estimated marginal efficiency of protein utilization. There are no clear indications that increasing protein intake increases ruminal drinking, and therefore has a significant impact on marginal protein efficiency. It is, however, clear that ruminal drinking can be quantitatively important, and an important source of between-animal variation.

6.2. Preferential utilization by particular tissues

Following digestion and absorption, amino acids are available for protein synthesis or oxidation. High rates of protein turnover of specific tissues, like the gut, liver, pancreas and kidney, could have affected the marginal efficiency of single amino acids as calculated by Gerrits et al. [43]. The contribution of tissues with a high turnover rate may be higher than can be expected based on their contribution to empty body protein, because high rates of protein turnover may be associated with increased oxidative losses [66]. Also, a specific preference of particular tissues to utilize indispensable amino acids can lead to an imbalance of amino acids at the site of deposition. The digestive tract, for example, can metabolize dietary indispensable amino acids in monogastrics [67] and ruminants [68]. We performed a small-scale study in which portal amino acid fluxes were measured in four preruminant calves (161 kg BW) fed clotting and non-clotting protein sources [54, 55]. The calves were surgically fitted with catheters in the portal vein, the carotid artery and the mesenteric vein and were assigned to a diet (20% crude protein) containing either skimmed milk protein or soy and wheat gluten. In a crossover design with two collection periods of two days each, portal and arterial blood

Table II. Net portal fluxes of amino acids as percentage of amino acid intake (milk-fed calf and milk-fed pig) or apparent ileal digestible amino acids (pig, dairy cow and sheep) for several farm animals.

Amino acid	Milk-fed calf[1]	Milk-fed pig[2]	Pig[3]	Dairy cow[4]	Sheep[5]
Threonine	86	50	64	43	72
Tryptophane	ND	ND	92	ND	ND
Methionine	86	59	80	67	85
Cysteine	ND	17	93	–162	–3
Valine	89	67	74	51	68
Isoleucine	85	74	83	62	75
Leucine	80	66	75	62	70
Lysine	91	52	87	55	80
Histidine	93	70	103	95	96
Phenylalanine	96	57	83	76	84
Tyrosine	98	132	88	ND	97
Arginine	150	142	102	63	89
Aspartic acid	50	28	48	9	49
Serine	101	71	98	75	117
Glutamic acid	3	–2	–132	9	–51
Glycine	87	61	75	42	134
Alanine	180	190	215	80	107
Proline	65	87	48	9	95

[1] [5]. [2] [69–72]. [3] [73–75]. [4] [76]. [5] [68, 77].

samples were taken after feeding of 1040 g milk replacer. The portal availability, as percentage of intake, of the indispensable amino acids was not affected by protein source ($P > 0.05$). Average values and comparable values for other farm animals from the literature are presented in Table II. A flawless comparison between species is hampered by different experimental conditions in conducted studies. The highly negative portal cysteine flux in the dairy cow (Tab. II), for example, was likely due to a methionine deficiency in that study. It does, however, provide some interesting leads. Firstly, the relatively high recovery of dietary indispensable amino acids in the portal vein of the milk-fed calf suggests that there is less amino acid sequestration in the portal drained viscera in preruminant calves than in the other species shown. This is presumably affected by the relatively high feeding level in the calf study. In pigs and beef steers, increasing the feeding level does not proportionally increase the irreversible loss rate over the portal drained viscera, which implies that portal amino acid recoveries increase with feed intake [78, 79]. Secondly, the absence of one poorly recovered indispensable amino acid in milk-fed calves indicates that the amino acid balance is not drastically disturbed by passing the portal drained viscera. Finally, portal arginine availability is high in milk-fed animals, both in calves and pigs. A major contribution of total arginine supply has to originate from endogenous synthesis in milk-fed animals, because milk supplies only about 60% of the arginine an animal needs for maximal growth [46, 80]. In neonatal pigs, the small intestine is the major site of endogenous arginine synthesis, but during development it becomes the major site of

citrulline production, which is converted to arginine by the kidney [81]. Studies on arginine metabolism have mainly been performed in milk-fed pigs during the first 21 d of life [46]. Arginine supplementation has been shown to increase growth in young calves [44, 45], but the mechanisms involved have not yet been studied. From this point of view, the high marginal efficiencies for arginine in heavy preruminant calves (97% for 80–160 kg BW; 89% for 160–240 kg BW) [43] may have some biological significance. Conversely, the low marginal efficiency of proline (~ 35%), which is a precursor for endogenous arginine synthesis, did not suggest any limitation of arginine availability. Arginine kinetics have not been studied in preruminant calves and quantification of arginine synthesis and the interaction with the ornithine cycle activity as well as identification of the tissue(s) involved would be interesting.

The liver is the second tissue which can be studied individually. Although several studies report hepatic nutrient fluxes in preruminant calves [82–85], the fate of individual amino acids is not described and amino acid catabolism by the liver can not be quantified. In conclusion, the few data available do not directly suggest preferential utilization of amino acids by specific tissues in preruminant calves.

6.3. Recycling of urea nitrogen

Recycling of urea by microbes in the gastrointestinal tract of dairy cows (67% of urea synthesis) and sheep (61% of urea synthesis) contributes to a large extent to the nitrogen supply in ruminant animals and can even exceed the apparent digestible nitrogen intake [86]. In milk-fed calves, this partial reuse of urea is expected to be negligible due to the absence of non-starch polysaccharides provoking microbial activity in the gastrointestinal tract. The recovery of an intravenous pulse dose of ^{13}C-urea was about 80% in 48 h urine of milk-fed calves [56] , which indicates that urea recycling is substantially lower than in rumi-

nants [86]. Provision of roughage (viz. potential carbon precursors) will definitely increase urea recycling and therefore the utilization of digestible protein for growth, provided that ruminal drinking is not promoted by the provision of roughage.

6.4. Utilization of amino acids for gluconeogenesis

Donkin and colleagues [87–89] have intensively studied gluconeogenesis in hepatocyte monolayers of milk-fed calves as affected by insulin and glucagon. They showed that the hepatic capacity for gluconeogenesis from lactate in milk-fed calves is much higher than in ruminant calves. Also, Ortigues et al. [85] suggested that the Cori cycle can be of greater importance in preruminants than in ruminants. For propionate, the gluconeogenic capacity was shown to be at least as high as in ruminant calves [88]. This substantial capacity for gluconeogenesis in preruminant calves seems to be redundant, because large amounts of monosaccharides are supplied by the diet. On the contrary, ruminating animals have an obligate need for gluconeogenesis, and it has been suggested that gluconeogenesis from amino acids is quantitatively important, even if the dietary glucose supply is abundant [90]. If genetically predisposed, preruminants potentially catabolize amino acids for gluconeogenesis, causing a low marginal efficiency of protein utilization.

In vivo quantification of gluconeogenesis and especially the contribution of different precursors (e.g. lactate, glycerol and amino acids), in preruminant calves is lacking. Therefore, despite the considerable gluconeogenic capacity and enzyme activity in milk-fed calves, the quantity of amino acids used for gluconeogenesis is not known.

6.5. Decreased insulin sensitivity

Heavy preruminant calves often develop a certain level of insulin resistance, indicated by postprandial hyperglycemia, hyperinsulinemia and glucosuria [91, 92]. The

etiology of insulin resistance in preruminant calves is likely to be multi-factorial, as reviewed by Blum and Hammon [93]. A high level of circulating insulin down-regulates its own receptors, which may result in altered cellular glucose and amino acid transport and compromised protein synthesis. Feeding large amounts of milk (especially lactose) to ontogenically ruminant animals, may contribute to the development of insulin resistance [93, 92]. In addition, increasing protein intake decreased insulin resistance, as indicated by glucosuria and high plasma concentrations of glucose and insulin, in preruminant calves [94]. Mechanistic explanations for the effect of protein intake on insulin resistance are not yet clarified. If insulin resistance coincides with reduced protein utilization, this effect will increase with increasing protein intake, thus contributing to a low marginal efficiency of protein utilization.

The consequences of insulin resistance for the utilization of amino acids in preruminant calves are, however, unknown. The effects of non insulin-dependent diabetes on protein utilization in human subjects are generally absent [95, 96], although Gougeon et al. [97] reported increased rates of protein breakdown and a more negative nitrogen balance in diabetic subjects. The low feeding level (non-growing man) and post-absorptive measurements complicate the extrapolation of these results to quickly growing milk-fed calves. Moreover, endocrine regulation of protein metabolism in the heavy preruminant calf is not unambiguous, because the responsiveness to insulin is apparently different in ruminants and non-ruminants [98–100]. Insufficient insight in the regulation of protein metabolism in preruminant calves raises the question if insulin resistance contributes to the low marginal efficiency of protein utilization.
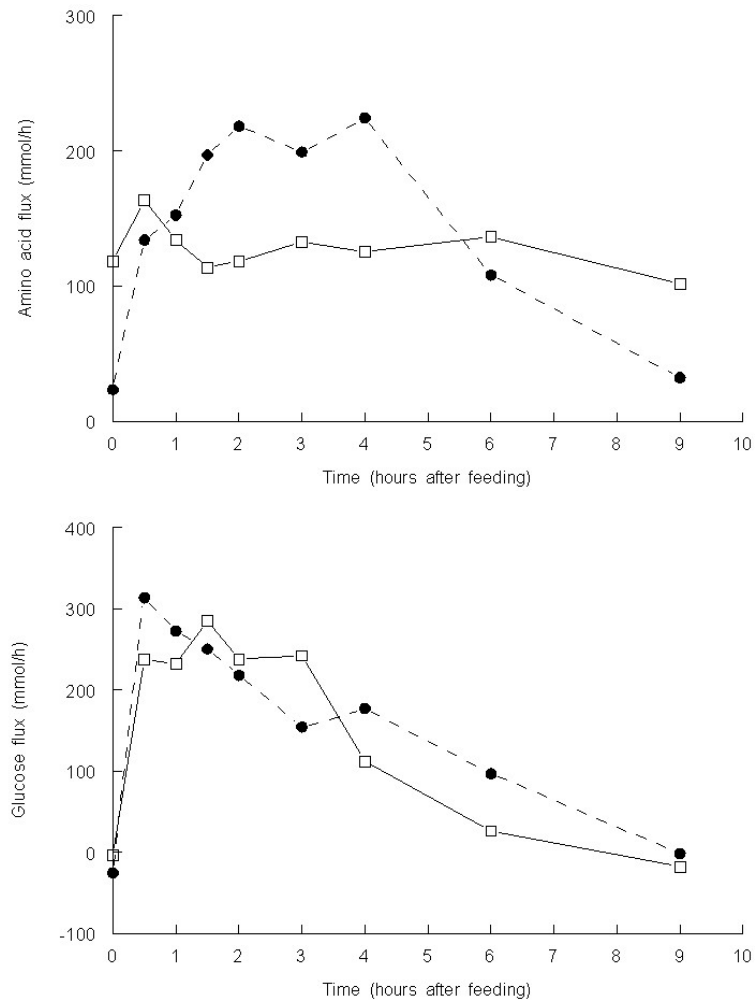
6.6. Post-absorptive nutrient asynchrony

Although all macronutrients are provided simultaneously to preruminant calves, an asynchronous post-absorptive availability of individual nutrients may occur. Separation of amino acid and glucose availability in time, for example, can be expected when skimmed milk protein is fed to calves. Casein (~ 80% of milk protein) clots in the calf abomasum and leads to a slow release of protein into the intestinal tract [5, 83, 101], while lactose does not have clotting abilities and is absorbed relatively quickly compared with dietary protein [5, 102] (Fig. 3). An asynchronous availability of glucose and amino acids within a day was shown to substantially decrease protein utilization in the adult man [reviewed by 103] and growing rats [104].

Apart from an asynchrony between individual nutrients, also an asynchrony between total nutrient supply and total nutrient requirements within a day may occur. Preruminant calves are usually fed twice daily, resulting in two peaks of metabolically available nutrients, while in ruminants, these patterns are absent because of eating patterns and gradual passage of feed through the rumen compartment. It can be hypothesized that in milk-fed calves, the inefficiency of protein utilization can to some extent be the consequence of a temporary excessive amino acid availability. In man, for example, it has been shown that protein retention increases with a slower digestion rate of dietary protein [105, 106]. The peak-wise glucose availability, however, requires flexibility of metabolic pathways to clear glucose from the systemic circulation. A disturbed glucose metabolism may also indirectly affect protein utilization (see previous section).

Finally, asynchrony occurs when individual amino acids are not available at the same moment. In calf milk replacers, vegetable protein sources (mainly soy, wheat, potato) have partly replaced skimmed milk protein during the past decades, with subsequent addition of crystalline amino acids to reach an ideal amino acid pattern for growth. Supplementation of the limiting amino acids as crystalline amino acids to

Figure 3. Net portal fluxes of plasma free α-amino acids and glucose in preruminant calves (160 kg BW; $n = 4$), fed diets based on either skimmed milk protein (□, ——) or vegetable proteins (●, – – –). Data from Verdonk et al. [54, 55].

the feed compensates this limitation and improves growth performance. Absorption kinetics of the supplemented free amino acids and the protein-bound amino acids, however, differ [107, 108] and may lead to an amino acid imbalance at the site of deposition.

Skimmed milk protein and respectively synthetic amino acids [36, 37] or caseinates

[31] were used as protein sources in the studies which demonstrated the low efficiency of protein utilization in preruminant calves. Therefore, the contribution of each type of nutrient asynchrony to the low marginal efficiency may have varied between studies. In general, interpretation of studies about nutrient asynchrony is complicated, since the three types of nutrient asynchrony

are often confounded. The use of a slowly digestible protein source [105], for example, results in a more gradual supply of amino acids during the day, but also in an increased asynchrony between amino acid and glucose absorption. Separate effects of different types of nutrient asynchrony can counteract, as shown by the interaction between protein source and feeding frequency on amino acid oxidation in pigs [109].

7. CONCLUSIONS AND IMPLICATIONS

It was determined that the utilization of extra dietary protein for protein gain is lower in preruminant calves than in several other species of farm animals. Especially preruminant calves above 100 kg BW showed a low marginal efficiency of protein utilization (< 30%). The inefficient utilization could not be explained by either a protein to energy imbalance or an imbalanced amino acid profile in the diet. Also the utilization of amino acids for ammonia detoxification did not contribute to the low efficiency. Alternative mechanisms result in either a reduced post-absorptive supply of amino acids or any indispensable amino acid (e.g. fermentation of milk or preferential utilization by tissues), or a reduced utilization of the post-absorptive available amino acids (e.g. insulin resistance or nutrient asynchrony). Nonetheless, the possibility of multiple factors being involved should not be excluded.

The low efficiency of protein utilization illustrates that there is a large potential for improvement of the efficiency of growth in heavy preruminant calves. More insight in the amino acid metabolism of preruminant calves is needed to decrease nitrogen losses. Furthermore, mechanisms of amino acid utilization in preruminant calves could also be relevant for ruminants in which similar studies are hampered by rumen fermentation processes.

REFERENCES

[1] Lallès JP, Toullec R, Bouchez P, Roger L. Antigenicity and digestive utilization of four soya products by the preruminant calf. Livest Prod Sci 1995, 41: 29–38.

[2] Le Huërou-Luron I, Gestin M, Le Dréan G, Romé V, Bernard C, Chayvialle JA, Guilloteau P. Source of dietary protein influences kinetics of plasma gut regulatory peptide concentration in response to feeding in preruminant calves. Comp Biochem Physiol A 1998, 119: 817–824.

[3] Montagne L, Salgado P, Toullec R, Lallès JP. Enzymes of the small intestine of the calf: Effect of dietary protein source on the activities of some enzymes in the small intestinal mucosa and digesta. J Sci Food Agric 2002, 82: 1772–1779.

[4] Toullec R, Guilloteau P. Research into the digestive physiology of the milk-fed calf. In: Van Weerden EJ, Huisman J (Eds), Nutrition and digestive physiology of monogastric farm animals, Pudoc, Wageningen, 1989, p 37–55.

[5] Verdonk JMAJ, Gerrits WJJ, Beynen AC. Replacement of milk protein by vegetable protein in milk replacer diets for veal calves: digestion in relation to intestinal health. In: Blok MC, Vahl HA, De Lange L, Van de Braak AE, Hemke G, Hessing M (Eds), Nutrition and health of the gastrointestinal tract, Wageningen Academic Publishers, Wageningen, 2002, p 183–198.

[6] Grizard J, Dardevet D, Papet I, Mosoni L, Patureau-Mirand P, Attaix D, Tauveron I, Bonin D, Arnal M. Nutrient regulation of skeletal muscle protein metabolism in animals. The involvement of hormones and substrates. Nutr Res Rev 1999, 39: 61–74.

[7] Chung TK, Baker DH. Methionine requirement of pigs between 5 and 20 kg body weight. J Anim Sci 1992, 70: 1857–1863.

[8] Gahl MJ, Crenshaw TD, Benevenga NJ. Diminishing returns in weight, nitrogen, and lysine gain of pigs fed six levels of lysine from three supplemental sources. J Anim Sci 1995, 73: 3177–3187.

[9] Markert W, Kirchgessner M, Roth FX. Bilanzstudien zur Reduzierung der N-Ausscheidung von Mastschweinen. J Anim Physiol Anim Nutr 1993, 70: 159–171.

[10] Mnilk B, Harris CI, Fuller MF. Lysine utilization by growing pigs: simultaneous measurement of protein accretion and lysine oxidation. Br J Nutr 1996, 75: 57–67.

[11] Knap PW. Time trends of Gompertz growth parameters in "meat-type" pigs. Anim Sci 2000, 70: 39–49.

[12] Freetly HC, Nienaber JA, Brown-Brandl T. Relationships among heat production, body weight, and age in Finn sheep and Rambouillet ewes. J Anim Sci 2002, 80: 825–832.

[13] Freetly HC, Nienaber JA, Leymaster KA, Jenkins TG. Relationships among heat production, body weight, and age in Suffolk and Texel ewes. J Anim Sci 1995, 73: 1030–1037.

[14] Calo LL, McDowell RE, Van Vleck LD, Miller PD. Parameters of growth of Holstein-Friesian bulls. J Anim Sci 1973, 37: 417–422.

[15] Batterham ES, Andersen LM, Baignent DR, White E. Utilization of ileal digestible amino acids by growing pigs: effect of dietary lysine concentration on efficiency of lysine retention. Br J Nutr 1990, 64: 81–94.

[16] Bikker P. Protein and lipid accretion in body components of growing pigs. PhD thesis, Animal Nutrition Group, Wageningen Agricultural University, Wageningen, The Netherlands, 1994.

[17] Campbell RG, Dunkin AC. The effects of energy intake and dietary protein on nitrogen retention, growth performance, body composition and some aspects of energy metabolism of baby pigs. Br J Nutr 1983, 49: 221–230.

[18] De Lange CFM, Gillis AM, Simpson GJ. Influence of threonine intake on whole-body protein deposition and threonine utilization in growing pigs fed purified diets. J Anim Sci 2001, 79: 3087–3095.

[19] Dourmad JY, Guillou D, Sève B, Henry Y. Response to dietary lysine supply during the finishing period in pigs. Livest Prod Sci 1996, 45: 179–186.

[20] Hennig U, Wünsche J, Meinl M, Borgmann E, Kreienbring F. The influence of graded protein supply at a high energy level on the fattening performance and the retention and utilisation of feed energy, protein and amino acids by female fattening pigs. 3. N retention and N and lysine metabolism determined by means of N balance and the analysis of the carcasses. Arch Anim Nutr 1982, 32: 637–649.

[21] Krick BJ, Boyd RD, Roneker KR, Beermann DH, Bauman DE, Ross DA, Maisinger DJ. Porcine somatotropin affects the dietary lysine requirement and net lysine utilization for growing pigs. J Nutr 1993, 123: 1913–1922.

[22] Möhn S, Gillis AM, Moughan PJ, De Lange CFM. Influence of dietary lysine and energy intakes on body protein deposition and lysine utilization in the growing pig. J Anim Sci 2000, 78: 1510–1519.

[23] Nieto R, Miranda A, García MA, Aguilera JF. The effect of dietary protein content and feeding level on the rate of protein deposition and energy utilization in growing Iberian pigs from 15 to 50 kg body weight. J Nutr 2002, 88: 39–49.

[24] Noblet J, Henry Y, Dubois S. Effect of protein and lysine levels in the diet on body gain composition and energy utilization. J Anim Sci 1987, 65: 717–726.

[25] Williams NH, Stahly TS, Zimmerman DR. Effect of chronic immune system activation on body nitrogen retention, partial efficiency of lysine utilization, and lysine needs of pigs. J Anim Sci 1997, 75: 2472–2480.

[26] Black JL, Pearce GR, Tribe DE. Protein requirements of growing lambs. Br J Nutr 1973, 30: 45–60.

[27] Phillips DD, Walker DM. Milk replacers containing isolated groundnut protein for preruminant lambs: the effect of protein concentration and energy intake on the requirement for lysine. Aust J Agric Sci 1980, 31: 133–145.

[28] Walker DM, Faichney GJ. Nitrogen balance studies with the milk-fed lamb. 3. Effect of different nitrogen intakes on growth and nitrogen balance. Br J Nutr 1964, 18: 295–306.

[29] Blome RM, Drackley JK, McKeith FK, Hutjens MF, McCoy GC. Growth, nutrient utilization, and body composition of dairy calves fed milk replacers containing different amounts of protein. J Anim Sci 2003, 81: 1641–1655.

[30] Donnelly PE, Hutton JB. Effects of dietary protein and energy on the growth of Friesian bull calves, I. Food intake, growth, and protein requirements. N Z J Agric Res 1976, 19: 289–297.

[31] Gerrits WJJ, Tolman GH, Schrama JW, Tamminga S, Bosch MW, Verstegen MWA. Effect of protein and protein-free energy intake on protein and fat deposition rates in preruminant calves of 80 to 240 kg live weight. J Anim Sci 1996, 74: 2129–2139.

[32] Tolman GH, Beelen GM. Endogenous nitrogen and amino acid flow in the terminal ileum of veal calves and the true digestibility of skim milk, soluble wheat and soya isolate proteins, In: Veal, Perspectives to the Year 2000, Proceedings of an International Symposium, Fédération de la Vitellerie Française, Le Mans, France, 1996, p 191–207.

[33] Keusenhoff R. Einflußfaktoren auf die N-Ausscheidungen beim Kalb. Arch Tierz 1992, 35: 571–579.

[34] Ternouth JH, Stobo JF, Roy JHB. The effect of milk substitute concentration upon the intake, digestion and growth of calves. Anim Prod 1985, 41: 151–159.

[35] Van Weerden EJ, Huisman J. Amino acid requirement of the young veal calf. J Anim Physiol Anim Nutr 1985, 53: 232–244.

[36] Tolman GH, Wiebenga J. The lysine and methionine+cystine requirement of Friesian veal calves in various weight ranges. Internal report nr I 91–3752A, TNO Nutrition and Food Research Institute, Dept. of Animal Nutrition and Meat Technology, Wageningen, The Netherlands, 1991 (in Dutch).

[37] Tolman GH, Wiebenga J, Beelen GM. The lysine and methionine+cystine requirement of Friesian veal calves (220–250 kg). Internal report nr I 91–3740, TNO Nutrition and Food Research Institute, Dept. of Animal Nutrition and Meat Technology, Wageningen, The Netherlands, 1991 (in Dutch).

[38] Tolman GH. The lysine, methionine+cystine and threonine requirement and utilization of non-ruminating veal calves of 50–70 kg. In: Nunes AF, Portugal AV, Costa JP, Ribeiro JR (Eds), Protein Metabolism and Nutrition, EAAP Publ No 81, Vale de Santarém, Portugal, 1996, p 273–274.

[39] Black JL, Campbell RG, Williams IH, James KJ, Davies GT. Simulation of energy and amino acid utilisation in the pig. Res Dev Agric 1986, 3: 121–145.

[40] Campbell RG, Taverner MR, Curic DM. Effects of sex and energy intake between 48 and 90 kg live weight on protein deposition in growing pigs. Anim Prod 1985, 40: 497–503.

[41] Dunkin AC, Black JL, James KJ. Relation between energy intake and nitrogen retention in entire male pigs weighing 75 kg. Br J Nutr 1986, 55: 201–207.

[42] Schroeder GF, Titgemeyer EC, Awawdeh MS, Gnad DP. Effects of energy supply on methionine utilization by growing steers. J Dairy Sci 2004, 87 (Suppl 1): 115.

[43] Gerrits WJJ, Schrama JW, Tamminga S. The marginal efficiency of utilization of all ileal digestible indispensible amino acids for protein gain is lower than 30% in preruminant calves between 80 and 240 kg live weight. J Nutr 1998, 128: 1774–1785.

[44] Fligger JM, Gibson CA, Sordillo LM, Baumrucker CR. Arginine supplementation increases weight gain, depresses antibody production, and alters circulating leukocyte profiles in preruminant calves without affecting plasma growth hormone concentrations. J Anim Sci 1997, 75: 3019–3025.

[45] Hüsler BR, Blum JW. Metabolic and endocrine changes in response to endotoxin administration with or without oral arginine supplementation. J Dairy Sci 2002, 85: 1927–1935.

[46] Kim SW, McPherson RL, Wu G. Dietary arginine supplementation enhances the growth of milk-fed young pigs. J Nutr 2004, 134: 625–630.

[47] Milano GD, Lobley GE. Liver nitrogen movements during short-term infusion of high levels of ammonia into the mesenteric vein of sheep. Br J Nutr 2001, 86: 507–513.

[48] Lobley GE, Milano GD. Regulation of hepatic nitrogen metabolism in ruminants. Proc Nutr Soc 1997, 56: 547–563.

[49] Orzechowsky A, Pierzynowski S, Motyl T, Barej W. Net hepatic metabolism of ammonia, propionate and lactate in sheep in relation to gluconeogenesis and ureagenesis. J Anim Physiol Anim Nutr 1988, 59: 113–122.

[50] Symonds HW, Mather DL, Collis KA. The maximum capacity of the liver of the adult dairy cow to metabolize ammonia. Br J Nutr 1981, 46: 481–486.

[51] Lobley GE, Weijs PJM, Connell A, Calder AG, Brown DS, Milne E. The fate of absorbed and exogenous ammonia as influenced by forage or forage-concentrate diets in growing sheep. Br J Nutr 1996, 76: 231–248.

[52] Luo QJ, Maltby SA, Lobley GE, Calder AG, Lomax MA. The effect of amino acids on the metabolic fate of $^{15}NH_4Cl$ in isolated sheep hepatocytes. Eur J Biochem 1995, 228: 912–917.

[53] Milano GD, Hotston-Moore A, Lobley GE. Influence of hepatic ammonia removal on ureagenesis, amino acid utilization and energy metabolism in the ovine liver. Br J Nutr 2000, 83: 307–315.

[54] Verdonk JMAJ, Gerrits WJJ, Beelen GM. Effect of protein source on portal nutrient fluxes in preruminant calves. Internal report No V99.030, ID TNO Animal Nutrition, Wageningen, The Netherlands, 2002 (in Dutch).

[55] Verdonk JMAJ, Gerrits WJJ, Beelen GM, Jansman AJM. Effect of protein source on portal nutrient fluxes in preruminant calves. In: Lobley GE, White A, MacRae JC (Eds), The VIIIth International Symposium on Protein Metabolism and Nutrition, Wageningen

Pers, The Netherlands, Aberdeen, UK, 1999, p 47.

[56] Gerrits WJJ, Beelen GM, Dijkstra J, Verdonk JMAJ. Ammonia infusion and starch fermentation in preruminant calves (150–180 kg). Internal report No V99.001, ID TNO Animal Nutrition, Wageningen, The Netherlands, 2001 (in Dutch).

[57] Gerrits WJJ, Dijkstra J, Verdonk JMAJ, Beelen GM, Boer H. Effects of ammonia and starch infusion in the colon of preruminant calves. In: Lobley GE, White A, MacRae JC (Eds), The VIIIth International Symposium on Protein Metabolism and Nutrition, Aberdeen, UK, 1999, p 55.

[58] Breukink HJ, Wensing T, Van Weeren-Keverling Buisman A, Van Bruinessen-Kapsenberg EG, De Visser NA. Consequences of failure of the reticular groove reflex in veal calves fed milk replacer. Vet Quart 1988, 10: 126–135.

[59] Gentile A, Sconza S, Lorenz I, Otranto G, Rademacher G, Famigli-Bergamini P, Klee W. Δ-Lactic acidosis in calves as a consequence of experimentally induced ruminal acidosis. J Vet Med Series A 2004, 51: 64–70.

[60] Rademacher G, Korn N, Friedrich A. The ruminal drinker as patient in practice. Tierärztl Umschau 2003, 58: 115–125 (in German).

[61] Guilhermet R, Mathieu CM, Toullec R. Transit des aliments liquides au niveau de la gouttière œsophagienne chez le veau préruminant et ruminant. Ann Zootech (Paris) 1975, 24: 69–79.

[62] Wise GH, Anderson GW, Linnerud AC. Relationship of milk intake by sucking and by drinking to reticular-groove reactions and ingestion behavior in calves. J Dairy Sci 1984, 67: 1983–1992.

[63] Tadeu dos Santos G, Toullec R, Roger R, De la Grange H, Guilloteau P. Caractéristiques digestives des veaux de boucherie s'adaptant mal en atelier d'engraissement. Reprod Nutr Dev 1986, 26: 1217.

[64] Van Leeuwen P. Duodenal passage of synthetic amino acids, dissolved in milk, when supplied six hours preprandial. Internal report nr 81.20A, TNO Nutrition and Food Research Institute, Dept. of Animal Nutrition and Meat Technology, Wageningen, The Netherlands, 1978 (in Dutch).

[65] Van Leeuwen P. Duodenal passage of synthetic amino acids, dissolved in water, when supplied three hours preprandial. Internal report No 81.20, TNO Nutrition and Food Research Institute, Dept. of Animal Nutrition and Meat Technology, Wageningen, The Netherlands, 1977 (in Dutch).

[66] Liu SM, Lobley GE, Macleod NA, Kyle DJ, Chen XB, Orskov ER. Effects of long-term protein excess or deficiency on whole-body protein turnover in sheep nourished by intragastric infusion of nutrients. Br J Nutr 1995, 73: 829–839.

[67] Burrin DG, Stoll B. Intestinal nutrient requirements in weanling pigs. In: Pluske JR, Le Dividich J, Verstegen MWA (Eds), Weaning the pigs – Concepts and consequences, Wageningen Academic Publishers, Wageningen, The Netherlands, 2003, p 301–335.

[68] MacRae JC, Bruce LA, Brown DS, Farningham DAH, Franklin M. Absorption of amino acids from the intestine and their net flux across the mesenteric- and portal-drained viscera of lambs. J Anim Sci 1997, 75: 3307–3314.

[69] Bos C, Stoll B, Fouillet H, Gaudichon C, Guan X, Grusak MA, Reeds PJ, Burrin DG, Tomé D. Postprandial intestinal and whole body nitrogen kinetics and distribution in piglets fed a single meal. Am J Physiol 2005, 288: E436–E446.

[70] Stoll B, Burrin DG, Henry J, Jahoor F, Reeds PJ. Phenylalanine utilization by the gut and liver measured with intravenous and intragastric tracers in pigs. Am J Physiol 1997, 273: G1208–G1217.

[71] Stoll B, Burrin DG, Henry J, Yu H, Jahoor F, Reeds PJ. Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. J Nutr 1998, 128: 1517–1524.

[72] Stoll B, Henry J, Reeds PJ, Yu H, Jahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J Nutr 1998, 128: 606–614.

[73] Lenis NP, Bikker P, Van der Meulen J, Van Diepen JTM, Bakker JGM, Jongbloed AW. Effect of dietary neutral detergent fiber on ileal digestibility and portal flux of nitrogen and amino acids and on nitrogen utilization in growing pigs. J Anim Sci 1996, 74: 2687–2699.

[74] Reverter M, Lundh T, Gonda HL, Lindberg JE. Portal net appearance of amino acids in growing pigs fed a barley-based diet with inclusion of three different forage meals. Br J Nutr 2000, 84: 483–494.

[75] Van der Meulen J, Bakker JGM, Smits B, De Visser H. Effect of source of starch on net portal flux of glucose, lactate, volatile fatty acids

and amino acids in the pig. Br J Nutr 1997, 78: 533–544.

[76] Berthiaume R, Dubreuil P, Stevenson M, McBride BW, Lapierre H. Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. J Dairy Sci 2001, 84: 194–203.

[77] Rémond D, Bernard L, Chauveau B, Nozière P, Poncet C. Digestion and nutrient net fluxes across the rumen, and the mesenteric- and portal-drained viscera in sheep fed with fresh forage twice daily: Net balance and dynamic aspects. Br J Nutr 2003, 89: 649–666.

[78] Lapierre H, Bernier JF, Dubreuil P, Reynolds CK, Farmer C, Oeullet DR, Lobley GE. The effect of intake on protein metabolism across splanchnic tissues in growing beef steers. Br J Nutr 1999, 81: 457–466.

[79] Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc Nat Acad Sci 2000, 97: 11620–11625.

[80] Williams AP, Hewitt D. The amino acid requirements of the preruminant calf. Br J Nutr 1979, 41: 311–319.

[81] Wu G, Morris SM. Arginine metabolism: nitric oxide and beyond. Biochem J 1998, 336: 1–17.

[82] Houlier ML, Patureau-Mirand P, Durand D, Bauchart D, Bayle G, Lefaivre J. Influence de la vitesse d'absorption des acides aminés sur leur bilan hépatique chez le veau préruminant. Reprod Nutr Dev 1990, 30: 135.

[83] Houlier ML, Patureau-Mirand P, Durand D, Bauchart D, Lefaivre J, Bayle G. Transport des acides aminés dans l'aire splanchnique par le plasma sanguin et le sang chez le veau préruminant. Reprod Nutr Dev 1991, 31: 399–410.

[84] Ortigues I, Martin C, Durand D. Circadian changes in net nutrient fluxes across the portal-drained viscera, the liver, and the hindquarters in preruminant calves. J Anim Sci 1996, 74: 895–907.

[85] Ortigues I, Martin C, Durand D, Vermorel M. Circadian changes in energy expenditure in the preruminant calf: whole animal and tissue level. J Anim Sci 1995, 73: 552–564.

[86] Lapierre H, Lobley GE. Nitrogen cycling in the ruminant: a review. J Dairy Sci 2001, 84 (Suppl E): E223–E236.

[87] Donkin SS, Armentano LE. Regulation of gluconeogenesis by insulin and glucagon in the neonatal bovine. Am J Physiol 1994, 266: R1229–R1237.

[88] Donkin SS, Armentano LE. Insulin and glucagon regulation of gluconeogenesis in preruminating and ruminating bovine. J Anim Sci 1995, 73: 546–551.

[89] Donkin SS, Bertics SJ, Armentano LE. Chronic and transitional regulation of gluconeogenesis and glyconeogenesis by insulin and glucagon in neonatal calf hepatocytes. J Anim Sci 1997, 75: 3082–3087.

[90] Lobley GE. Control of the metabolic fate of amino acids in ruminants: a review. J Anim Sci 1992, 70: 3264–3275.

[91] Doppenberg J, Palmquist DL. Effect of dietary fat level on feed intake, growth, plasma metabolites and hormones of calves fed dry or liquid diets. Livest Prod Sci 1991, 29: 151–158.

[92] Hostettler-Allen RL, Tappy L, Blum JW. Insulin resistance, hyperglycemia, and glucosuria in intensively milk-fed calves. J Anim Sci 1994, 75: 160–173.

[93] Blum JW, Hammon HM. Endocrine and metabolic aspects in milk-fed calves. Domest Anim Endocrinol 1999, 17: 219–230.

[94] Gerrits WJJ, Blum JW. A role of protein intake in the development of insulin resistance in preruminant calves. In: Blum JW, Elsasser T, Guilloteau P (Eds), Symposium on Growth in Ruminants: Basic Aspects, Theory and Practice for the Future, Berne, Switzerland, 1998, p 310.

[95] Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Sabadin L, Fongher C, Panebianco G, Fratton MG, Tiengo A. Fasting and postmeal phenylalanine metabolism in mild type 2 diabetes. Am J Physiol 1992, 263: E877–E883.

[96] Luzi L, Petrides AS, De Fronzo RA. Different sensitivity of glucose and amino acid metabolism to insulin in NIDDM. Diabetes 1993, 42: 1868–1877.

[97] Gougeon R, Pencharz PB, Marliss EB. Effect of NIDDM on the kinetics of whole-body protein metabolism. Diabetes 1994, 43: 318–328.

[98] Garlick PJ, Grant I. Amino acid infusion increased the sensitivity of muscle protein synthesis in vivo to insulin: effect of branch chain amino acids. Biochem J 1988, 254: 579–584.

[99] Oddy VH, Lindsay DB, Barker PJ, Northrop AJ. Effect of insulin on hind limb and whole-body leucine and protein metabolism in fed and fasted lambs. Br J Nutr 1987, 58: 143–154.

[100] Wester TJ, Lobley GE, Birnie LM, Crompton LA, Brown DS, Buchan V, Calder AG, Milne E, Lomax MA. Effect of plasma insulin and branched-chain amino acids on skeletal muscle protein synthesis in fasted lambs. Br J Nutr 2004, 92: 401–409.

[101] Longenbach JI, Heinrichs AJ. A review of the importance and physiological role of curd formation in the abomasum of young calves. Anim Feed Sci Technol 1997, 73: 85–97.

[102] Grizard J, Toullec R, Guilloteau P, Patureau-Mirand P. Effect of the kinetics of gastric emptying of food on blood insulin levels in the preruminant calf. Reprod Nutr Dev 1982, 22: 475–484 (in French).

[103] Munro HN. Carbohydrate and fat as factors in protein utilization and metabolism. Physiol Rev 1951, 31: 449–488.

[104] Geiger E. The importance of the time element in feeding of growing rats. Experiments with delayed supplementation of protein. Science 1948, 108: 42–43.

[105] Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Nat Acad Sci 1997, 94: 14930–14935.

[106] Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O, Beaufrère B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol 2001, 280: E340–E348.

[107] Metges CC, El-Khoury AE, Selvaraj AB, Tsay RH, Atkinson A, Regan MM, Bequette BJ, Young VR. Kinetics of L-[1-^{13}C]leucine when ingested with free amino acids, unlabeled or intrinsically labelled casein. Am J Physiol 2000, 278: E1000–E1009.

[108] Yen JT, Kerr BJ, Easter RA, Parkhurst AM. Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily. J Anim Sci 2004, 82: 1079–1090.

[109] Batterham ES, Bayley HS. Effect of frequency of feeding of diets containing free or protein-bound lysine on the oxidation of [^{14}C]lysine or [^{14}C]phenylalanine by growing pigs. Br J Nutr 1989, 62: 647–655.

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